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Sibling competition does not exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*

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Abstract

Inbreeding results from matings between relatives and can cause a reduction in offspring fitness, known as inbreeding depression. Previous work has shown that a wide range of environmental stresses, such as extreme temperatures, starvation, and parasitism, can exacerbate inbreeding depression. It has recently been argued that stresses due to intraspecific competition should have a stronger effect on the severity of inbreeding depression than stresses due to harsh physical conditions. Here, we tested whether an increase in the intensity of sibling competition can exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*. We used a 2×3 factorial design with offspring inbreeding status (outbred or inbred) and brood size (5, 20, or 40 larvae) as the two factors. We found a main effect of inbreeding status, as inbred larvae had lower survival than outbred larvae, and a main effect of brood size, as larvae in large broods had lower survival and mass than larvae in medium-sized broods. However, there was no effect of the interaction between inbreeding status and brood size, suggesting that sibling competition did not influence the severity of inbreeding depression. Since we focused on sibling competition within homogeneous broods of either inbred or outbred larvae, we cannot rule out possible effects of sibling competition on inbreeding depression in mixed paternity broods comprising of both inbred and outbred offspring. More information on whether and when sibling competition might influence the expression of inbreeding depression can help advance our understanding of the causes underlying variation in the severity of inbreeding depression.

Keywords: burying beetle, environmental stress, fitness, inbreeding depression, *Nicrophorus vespilloides*, sibling competition

Introduction

Inbreeding results from matings between relatives and can cause a reduction in offspring fitness, known as inbreeding depression (Crnokrak & Roff, 1999). These negative fitness effects are due to the higher degree of homozygosity associated with inbreeding, which increases the risk that deleterious recessive alleles are expressed (Charlesworth & Charlesworth, 1987). Although there is widespread evidence for inbreeding depression in a range of taxa (Crnokrak & Roff, 1999), there is substantial variation both among and within species with respect to the severity of inbreeding depression (Crnokrak & Roff, 1999; Keller & Waller, 2002; Meunier & Kölliker, 2013; Moorad & Wade, 2005). This variation may be in part driven by differences in the physical and social environment, which can have profound effects on inbreeding depression (Fox & Reed, 2011; Reed *et al*., 2012). For example, environmental stresses, such as extreme temperatures, starvation, parasitism, and competition, can exacerbate inbreeding depression (Fox & Reed, 2011; Meunier & Kölliker, 2013), while parental care, which evolved to neutralize environmental stresses to the offspring, can buffer against inbreeding depression (Avilés & Bukowski, 2006; Pilakouta *et al*., 2015a). Although there is growing evidence that physical and social stresses can alter the severity of inbreeding depression (Fox & Reed, 2011; Reed *et al.*, 2012), little is known about the mechanisms by which particular environmental stresses influence its expression.

A recent study suggested that stresses due to intense intraspecific competition over limited resources should have a stronger effect on the severity of inbreeding depression than stresses due to harsh physical conditions, such as extreme temperatures (Yun & Agrawal, 2014). Intraspecific competition over limited resources can take several forms and can occur at different stages of the life cycle, including competition with siblings during development (Mock & Parker, 1997). Sibling competition for resources provided by the parents occurs because parents usually produce an optimistic brood size, thereby creating a mismatch

between the supply of resources from the parents and the demand of resources by the offspring (Mock & Parker, 1997). Sibling competition is an important determinant of the offspring's growth and survival in many species and may therefore be a key source of environmental stress to the offspring (Mock & Parker, 1997; Roulin & Dreiss, 2012; Meunier & Kölliker, 2013). Thus, sibling competition should be associated with an increase in this mismatch between supply and demand of resources, which in turn may exacerbate inbreeding depression. To our knowledge, the only experimental study to test this hypothesis was conducted on the European earwig, *Forficula auricularia* (Meunier & Kölliker, 2013). This study found no effect of the interaction between the intensity of sibling competition and the offspring's inbreeding status on offspring fitness, suggesting that sibling competition did not influence the severity of inbreeding depression (Meunier & Kölliker, 2013). However, the absence of such an interaction effect may reflect that there was no evidence for a main effect of inbreeding status on offspring fitness during the early life stages (Meunier & Kölliker, 2013). In order to advance our understanding of whether sibling competition can exacerbate inbreeding depression, it is now essential to focus on species in which inbred offspring suffer a significant reduction in fitness and sibling competition negatively affects offspring fitness.

Here, we tested whether sibling competition influences the severity of inbreeding depression in the burying beetle *Nicrophorus vespilloides*. This species is well suited for addressing this question, because previous work has shown that inbred offspring suffer significant fitness costs during the larval stage (Mattey *et al.*, 2013; Pilakouta *et al*., 2015a) and that larvae in experimentally enlarged broods suffer a reduction in fitness due to the increased mismatch between supply and demand for resources (Smiseth *et al.*, 2007a). In this species, which breeds on carcasses of small vertebrates, larvae compete for resources by begging for predigested carrion from the parents and by self-feeding directly from the carcass (Smiseth *et al., 2*003). Earlier work has also shown that sibling competition reduces offspring

fitness only in the presence of the parents, reflecting interference competition due to successful larvae excluding their siblings from getting access to the parents (Smiseth *et al.*, 2007a,b). Although the independent effects of inbreeding status and sibling competition on offspring fitness are well established in this species, there is no prior information on the effect of their interaction.

To test for such an interaction, we used a 2×3 factorial design with offspring inbreeding status and brood size as the two factors. We assessed the joint effects of inbreeding status and sibling competition on fitness traits previously shown to be affected by these two factors (Smiseth *et al.*, 2007a; Mattey *et al*., 2013; Pilakouta *et al*., 2015a): (*i*) average larval mass at dispersal; (*ii*) survival from hatching to dispersal; (*iii*) survival from dispersal to eclosion; and (*iv*) total survival from hatching to eclosion. If sibling competition exacerbates inbreeding depression, we would expect offspring in larger broods to incur higher fitness costs if they are inbred than if they are outbred.

Materials and methods

Study species

Like all burying beetles of the genus *Nicrophorus*, *N. vespilloides* breeds on carcasses of small vertebrates and has highly elaborate forms of parental care (Scott 1998). Once a carcass is found, parents bury it into the soil, remove any fur or feathers, deposit antimicrobial secretions to its surface, and lay eggs around it 24-48 hours after mating (Eggert, 1992; Scott, 1998). When the eggs hatch approximately 60 hours later (Smiseth *et al.*, 2006), the larvae crawl to the carcass and start feeding in a crater created by the parents on the top of the carcass. The larvae can self-feed, but the parents also provision larvae with predigested carrion (Smiseth *et al*., 2003). Although both parents typically provide care, females often stay on the carcass for longer than males and spend more time provisioning food to the larvae

(Fetherston *et al*., 1994; Eggert *et al*., 1998; Smiseth & Moore, 2002; Rauter & Moore, 2004; Smiseth *et al*., 2005; Pilakouta *et al.,* 2015b). Larvae disperse from the carcass about five days after hatching, which corresponds to the end of the parental care period. They eclose as adults about 20 days later.

Experimental design

We used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. The beetles used in this study comprised of sixth-, seventh-, and eighth-generation beetles from lines originally collected in Edinburgh, UK and Warmond, The Netherlands. Beetles were housed individually in transparent plastic containers (12×8×2 cm) filled with moist soil and kept at 20°C and constant light. Non-breeding adults were fed raw organic beef twice a week.

To test for a causal effect of sibling competition on the severity of inbreeding depression, we used a 2×3 factorial design with offspring inbreeding status (outbred or inbred) and brood size (5, 20, or 40 larvae) as the two factors. Inbred larvae were produced by pairing males and females that were full siblings (n=186), while outbred larvae were produced by pairing unrelated males and females that shared no common ancestors for at least two generations (n=187). We only used outbred parents in this experiment as inbreeding in the parents has a negative effect on offspring survival (Mattey *et al*., 2013). These breeding pairs (n=373) were transferred to transparent plastic containers (17cm×12cm×6cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (22–25 g). Immediately after the eggs were laid, we removed the male and moved the female and the carcass to a new container with fresh, moist soil. We removed the males because the amount of care provided by the male is highly variable and male removal has no effect on offspring fitness under

laboratory conditions (Smiseth *et al*., 2005). We left the females to provide care for the brood because previous work on this species showed that sibling competition reduces offspring fitness only when larvae compete by begging for food from a parent (Smiseth *et al.,* 2007a,b).

When the eggs started hatching, we used the newly hatched larvae to generate inbred and outbred broods comprising of 5, 20, or 40 larvae. All experimental broods included larvae of mixed maternity in accordance with established protocols (Smiseth *et al*., 2007; Pilakouta *et al*., 2015b). This brood size manipulation is within the natural variation of brood size in *N. vespilloides* (mean ± SD: 21 ± 10 larvae, range: 2–47 larvae; Smiseth & Moore, 2002) and corresponds to small, average, and large broods (i.e., low, medium, and high level of sibling competition), respectively. We used a design with more than two levels of stress, because the relationship between stress and inbreeding depression may be nonlinear (Fox & Reed, 2011).

Each experimental brood (outbred or inbred) was randomly assigned to an unrelated female who had been mated either to their full-sib brother or to an unrelated male. To account for potential effects of relatedness between the female and her male partner (who was always removed before the female was provided with a foster brood), we added this information as a factor in all of our models (see below). In this species, parents cannot distinguish between unrelated foster broods and their own broods, as long as the larvae are at the same developmental stage (Müller & Eggert, 1990). Since parents kill any larvae that arrive on the carcass before their eggs are expected to hatch (Müller & Eggert, 1990), we only provided females with a brood once their own eggs had hatched.

Females were left to care for their brood until the larvae dispersed from the carcass about five days later. At dispersal from the carcass, we recorded the number of larvae and total brood mass to calculate larval survival rate and average larval mass. Lastly, to assess survival after independence (i.e., from dispersal to eclosion), we placed all dispersing larvae

from each brood into a large transparent container (17cm×12cm×6cm) filled with moist soil. About 20 days after dispersal, we recorded the number of individuals that eclosed successfully from each brood and calculated the survival rate from dispersal to eclosion.

The total sample size in the experiment was n=166 broods. The sample sizes for the different treatments were as follows: n=31 for outbred broods with 5 larvae, n=32 for outbred broods with 20 larvae, n=22 for outbred broods with 40 larvae, n=31 for inbred broods with 5 larvae, n=30 for inbred broods with 20 larvae, and n=20 for inbred broods with 40 larvae.

Statistical analysis

Data were analyzed using R version 3.2.0. Larval mass at dispersal was analyzed using a linear model. Proportion data (i.e., survival rates from hatching to dispersal, from dispersal to eclosion, and from hatching to eclosion) were analyzed using generalized linear models fitted with a binomial distribution corrected for overdispersion. Proportion data were entered into the models using the 'cbind' function.

All models included offspring inbreeding status (outbred or inbred), brood size (small, medium, or large), and the interaction between these two factors. As additional factors, we included carcass mass, the relatedness between the foster mother and her removed male partner (i.e., whether the female rearing the brood had been mated to a full sibling or an unrelated male), the interaction between foster parent relatedness and offspring inbreeding status, and the interaction between foster parent relatedness and brood size. Decisions on which factors to include in the final models were based on AIC scores. For models where brood size was found to have a statistically significant effect, we used the 'glht' function in the 'multcomp' package (Hothorn *et al*. 2008) to perform a Tukey test for post-hoc pairwise comparisons.

Results

We found evidence for a main effect of offspring inbreeding status on survival to dispersal, survival to eclosion, and total survival, as inbred larvae suffered lower survival than outbred larvae (Table 1; Figure 1b-d). There was no significant difference in average larval mass between inbred and outbred larvae (Table 1; Figure 1a). In addition, we found evidence for a main effect of sibling competition on offspring fitness: larvae in large broods were smaller and had a lower rate of survival to dispersal than larvae in medium-sized broods (Tables 1 and 2; Figure 1a-b). Sibling competition also had a non-significant effect on total survival (Tables 1 and 2; Figure 1d). In contrast, larvae in small broods had a lower rate of survival to eclosion than larvae in medium-sized or large broods (Table 2; Figure 1c). We found no evidence that sibling competition exacerbated inbreeding depression, as there was no effect of the interaction between the offspring's inbreeding status and the size of the brood on any component of offspring fitness (Table 1). In addition, there was no effect of foster parent relatedness (i.e. whether the foster mother had been mated to a brother or an unrelated male) or the interaction between foster parent relatedness and offspring inbreeding status on offspring fitness (Table S1). There was an effect of the interaction between foster parent relatedness and brood size on survival to eclosion but not on larval mass, survival to dispersal, or total survival (Table S1). Lastly, larvae reared on larger carcasses had a higher larval mass at dispersal and higher overall survival (Table S1).

Discussion

We find no evidence for an effect of the interaction between sibling competition and inbreeding status, suggesting that sibling competition does not exacerbate inbreeding depression in *N. vespilloides*. We show that inbreeding status negatively affected offspring fitness, as inbred larvae suffered lower survival during both the larval and pupal stages.

These results demonstrate that there is significant inbreeding depression in this species as reported in previous studies (Mattey *et al*., 2013; Pilakouta *et al*., 2015a). Furthermore, we show that sibling competition reduced offspring fitness, as larvae in large broods were smaller and suffered lower survival to dispersal than larvae in medium-sized broods. These results confirm that the intensity of sibling competition has a negative effect on larval fitness as previously reported by Smiseth *et al*. (2007a). Even though there were significant main effects of both inbreeding status and sibling competition, there was no effect of the interaction between these two factors on offspring fitness. To our knowledge, the only other study to investigate this question (conducted on the European earwig) found no evidence for a main effect of inbreeding status on offspring fitness in the early life stages (Meunier & Kölliker, 2013). Given the absence of inbreeding depression, it was not possible to test whether inbreeding depression becomes more severe when sibling competition increases. Thus, our study is the first to show that sibling competition for resources provided by the parents does not exacerbate inbreeding depression in a species where inbred offspring do suffer substantial fitness costs.

There is accumulating evidence that the negative effects of inbreeding can be intensified by a wide range of environmental stresses, such as parasitism, starvation, population density, extreme temperatures, and exposure to chemicals (Armbruster & Reed, 2005; Waller *et al*., 2008; Fox & Reed, 2011; Reed *et al*., 2012). Nevertheless, we still have a limited understanding of the mechanisms by which particular stresses exacerbate inbreeding depression. Yun and Agrawal (2014) argue that density-dependent stresses caused by intense competition among conspecifics should have a stronger effect on the severity of inbreeding depression than density-independent stresses caused by harsh physical conditions. In their study on *Drosophila melanogaster*, they found a moderate correlation between environmental

stress and density dependence, but inbreeding depression was significantly more correlated with density dependence than environmental stress *per se* (Yun & Agrawal, 2014).

We expected that an increase in sibling competition should affect the severity of inbreeding depression because sibling competition is a density-dependent source of environmental stress caused by a mismatch between the supply and demand for resources (Mock & Parker, 1997; Roulin & Dreiss, 2012). Yet, we find that sibling competition does not exacerbate inbreeding depression in *N. vespilloides*, which appears to contradict the argument made by Yun and Agrawal (2014). One potential explanation for this discrepancy is the difference in experimental designs between our study and that of Yun and Agrawal (2014). We studied the effects of sibling competition within homogeneous broods comprised of either outbred or inbred larvae, while Yun and Agrawal (2014) studied the effects of competition within heterogeneous groups of unrelated inbred and outbred fruit flies. Thus, in our study, any effect of sibling competition on the severity of inbreeding depression would be mediated through an increase in the level of stress. In contrast, in the latter study, such effects would be mediated through direct competitive interactions between inbred individuals (inferior competitors) and outbred individuals (superior competitors). Homogeneous broods comprised of either outbred or inbred larvae are likely to be the norm in *N. vespillodes* given that caring males sire over 90% of the offspring in their brood (Müller & Eggert, 1989). In such homogeneous broods, all larvae may be equally disadvantaged by stresses caused by a shortage of resources. However, we cannot rule out possible effects of sibling competition on the severity of inbreeding depression in mixed paternity broods comprising of both inbred and outbred larvae. Under those conditions, higher-quality (i.e., outbred) offspring may outcompete their lower-quality (i.e., inbred) half-siblings, thus magnifying differences in fitness between them. We encourage future studies to investigate this question in the context of family groups that comprise of both outbred and inbred offspring, as in socially

monogamous birds where the female is closely related to her social partner and has extra-pair matings with non-relatives (e.g., Blomqvist *et al*., 2002; Foerster *et al*., 2003; Brouwer *et al*., 2011; Reid *et al*., 2015).

Even though understanding the factors that drive the observed variation in the severity of inbreeding depression across species and across environments could have important implications for the conservation of many endangered populations, these dynamics are still not well understood. Our findings suggest that the intensity of sibling competition may not contribute towards variation in the severity of inbreeding depression, at least within homogeneous broods of inbred offspring. Determining whether and when sibling competition might play a role in the expression of inbreeding depression may help us better understand the causes underlying temporal and spatial patterns of variation in inbreeding depression in natural populations.

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References

Armbruster, P. & Reed, D.H. 2005. Inbreeding depression in benign and stressful environments. *Heredity* **95:** 235–242.

Avilés, L. & Bukowski, T.C. 2006. Group living and inbreeding depression in a subsocial spider. *Proc. Biol. Sci.* **273:** 157–163.

288 Blomqvist, D., Andersson, M., Kupper, C., Cuthill, I.C., Kis, J., Lanctot, R.B., Sandercock,
289 B.K., Székely, T., Wallander, J. & Kempenaers, B. 2002 Genetic similarity between
290 mates and extra-pair parentage in three species of shorebird. *Nature* **419:** 613–615.
291 Brouwer, L., van de Pol, M., Atema, E. & Cockburn, A. 2011. Strategic promiscuity helps
292 avoid inbreeding at multiple levels in a cooperative breeder where both sexes are
293 philopatric. *Mol. Ecol.* **20:** 4796–4807.
294 Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary
295 consequences. *Annu. Rev. Ecol. Syst.* **18:** 237–268.
296 Crnokrak, P. & Roff, D. 1999. Inbreeding depression in the wild. *Heredity* **83:** 260–270.
297 Eggert, A.-K. 1992. Alternative male mate-finding tactics in burying beetles. *Behav. Ecol.* **3:**
298 243–254.
299 Eggert, A.-K., Reinking, M. & Müller, J.K. 1998. Parental care improves offspring survival
300 and growth in burying beetles. *Anim. Behav.* **55:** 97–107.
301 Fetherston, I.A., Scott, M.P. & Traniello, J.F.A. 1994. Behavioural compensation for mate
302 loss in the burying beetle *Nicrophorus orbicollis*. *Anim. Behav.* **47:** 777-785.
303 Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J.T. & Kempenaers, B. 2003. Females increase
304 offspring heterozygosity and fitness through extra-pair matings. *Nature* **425:** 714–717.
305 Fox, C.W. & Reed, D.H. 2011. Inbreeding depression increases with environmental stress: an
306 experimental study and meta-analysis. *Evolution* **65:** 246–258.
307 Hothorn, T., Bretz F. & Westfall, P. 2008. Simultaneous inference in general parametric
308 models. *Biometrical Journal* **50:** 346–363.
309 Keller, L. & Waller, D. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17:**
310 230–241.

311 Mattey, S.N., Strutt, L. & Smiseth, P.T. 2013. Intergenerational effects of inbreeding in
312 *Nicrophorus vespilloides*: offspring suffer fitness costs when either they or their parents
313 are inbred. *J. Evol. Biol.* **26:** 843–853.
314 Meunier, J. & Kölliker, M. 2013. Inbreeding depression in an insect with maternal care:
315 influences of family interactions, life stage and offspring sex. *J. Evol. Biol.* **26:** 2209-
316 2220.
317 Mock, D.W. & Parker, G.A. 1997. The evolution of sibling rivalry. Oxford University Press.
318 Moorad, J.A. & Wade, M.J. 2005. A genetic interpretation of the variation in inbreeding
319 depression. *Genetics* **170:** 1373-1384.
320 Müller, J.K. & Eggert, A.-K. 1989. Paternity assurance by "helpful" males: adaptations to
321 sperm competition in burying beetles. *Behav. Ecol. Soclobiol.* **24:** 245-249.
322 Müller, J.K. & Eggert, A.-K. 1990. Time-dependent shifts between infanticidal and parental
323 behavior in female burying beetles: a mechanism of indirect mother-offspring
324 recognition. *Behav. Ecol. Sociobiol.* **27:** 11-16.
325 Pilakouta, N., Jamieson, S., Moorad, J.A. & Smiseth, P.T.S. 2015. Parental care buffers
326 against inbreeding depression in burying beetles. *Proc. Natl. Acad. Sci. U.S.A.* **112:**
327 8031-8035.
328 Pilakouta, N., Richardson, J. & Smiseth, P.T.S. 2015. State-dependent cooperation in burying
329 beetles: parents adjust their contribution towards care based on both their own and their
330 partner's size. *J. Evol. Biol*. (doi: 10.1111/jeb.12712)
331 Rauter, C.M. & Moore, A.J. 2004. Time constraints and trade-offs among parental care
332 behaviours: effects of brood size, sex and loss of mate. *Anim. Behav.* **68:** 695-702.
333 Reed, D.H., Fox, C.W., Enders, L.S. & Kristensen, T.N. 2012. Inbreeding-stress interactions:
334 evolutionary and conservation consequences. *Ann. N.Y. Acad. Sci.* **1256:** 33-48.

335 Reid, J.M., Arcese, P., Keller, L.K., Germain, R.R., Duthie, A.B., Losdat, S., Wolak, M.E. &
336 Nietlisbach, P. 2015. Quantifying inbreeding avoidance through extra-pair
337 reproduction. *Evolution* **69:** 59-74.
338 Roulin, A. & Dreiss, A.N. 2012. Sibling competition and cooperation over parental care. In:
339 The Evolution of Parental Care (N.J. Royle, P.T. Smiseth, M. Kolliker, eds), pp. 133–
340 149. Oxford University Press, Oxford.
341 Scott, M.P. 1998. The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43:**
342 595–618.
343 Smiseth, P.T., Darwell, C. & Moore, A.J. 2003. Partial begging: an empirical model for the
344 early evolution of offspring signalling. *Proc. R. Soc. B* **270:** 1773–1777.
345 Smiseth, P.T., Dawson, C., Varley, E. & Moore, A.J. 2005. How do caring parents respond to
346 mate loss? Differential response by males and females. *Anim. Behav.* **69:** 551–559.
347 Smiseth, P.T., Lennox, L. & Moore, A.J. 2007a. Interaction between parental care and sibling
348 competition: parents enhance offspring growth and exacerbate sibling competition.
349 *Evolution* **61:** 2331-2339.
350 Smiseth, P.T. & Moore, A.J. 2002. Does resource availability affect offspring begging and
351 parental provisioning in a partially begging species? *Anim. Behav.* **63:** 577–585.
352 Smiseth, P.T., Ward, R.S.J. & Moore, A.J. 2006. Asynchronous hatching in *Nicrophorus*
353 *vespilloides*, an insect in which parents provide food for their offspring. *Funct Ecol* **20:**
354 151–156.
355 Smiseth, P.T., Ward, R.S.J. & Moore, A.J. 2007b. Parents influence asymmetric sibling
356 competition: experimental evidence with partially dependent young. *Ecology* **88:** 3174–
357 3182.
358 Waller, D.M., Dole, J. & Bersch, A.J. 2008. Effects of stress and phenotypic variation on
359 inbreeding depression in *Brassica rapa*. *Evolution* **62:** 917–931.

360 Yun, L. & Agrawal, A.F. 2014. Variation in the strength of inbreeding depression across
361 environments: effects of stress and density dependence. *Evolution* **68-12:** 3599–3606.
362

363 **Table 1.** Effects of offspring inbreeding status (inbred or outbred) and sibling competition
364 (small, medium-sized, or large brood) on offspring fitness traits: average larval mass at
365 dispersal (mg), survival rate from hatching to dispersal, survival rate from dispersal to
366 eclosion, and survival rate from hatching to eclosion.

	Larval mass (mg)		Survival to dispersal		Survival to eclosion		Total survival	
	F-value	*P*-value	LR χ^2	*P*-value	LR χ^2	*P*-value	LR χ^2	*P*-value
Inbreeding status	2.27	0.13	4.54	**0.03**	37.79	**<0.0001**	17.17	**<0.0001**
Brood size	4.93	**<0.01**	6.72	**0.03**	12.23	**<0.01**	6.07	**0.048**
Interaction	0.09	0.91	0.31	0.86	4.00	0.14	0.38	0.83

367

368 Larval mass data were analyzed using a linear model. Survival rate data were analyzed using
369 generalized linear models fitted with a binomial distribution corrected for overdispersion. LR
370 refers to likelihood ratio. Statistically significant *P*-values are in bold.

Table 2. Post-hoc pairwise comparisons (Tukey test) for the effect of sibling competition (small, medium-sized, or large brood) on offspring fitness traits: average larval mass at dispersal (mg), survival rate from hatching to dispersal, survival rate from dispersal to eclosion, and survival rate from hatching to eclosion.

	Larval mass (mg)				Survival to dispersal				Survival to eclosion				Total survival			
Brood size	Est	SE	*t*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*
Small-Medium	-12.37	5.75	-2.15	0.08	-0.10	0.26	-0.38	0.92	-1.15	0.34	-3.36	**<0.01**	-0.49	0.25	-1.97	0.11
Small-Large	6.65	6.38	1.04	0.55	0.29	0.25	1.13	0.49	-1.13	0.33	-3.40	**<0.01**	-0.18	0.24	-0.73	0.74
Medium-Large	19.02	6.39	2.98	**<0.01**	0.39	0.15	2.52	**0.03**	0.02	0.27	-0.06	>0.99	0.31	0.15	2.08	0.09

We provide information on the parameter estimates (Est), standard errors (SE), test statistics (*t*- and *z*-values), and *P*-values. Parameter estimates represent the difference when subtracting the mean fitness of larvae in the second group listed from the mean fitness of larvae in the first group on each row. Statistically significant *P*-values are in bold.